PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 21 August 2000 (21.08.00)	
International application No. PCT/GB99/04395	**Applicant's or agent's file reference** PA9847
International filing date (day/month/year) 23 December 1999 (23.12.99)	**Priority date** (day/month/year) 30 December 1998 (30.12.98)
Applicant KNOX, Peter et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

19 July 2000 (19.07.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Pascal Piriou

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PA9847		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/04395	23/12/1999	30/12/1998
International Patent Classification (IPC) or national classification and IPC G01N33/58		
Applicant NYCOMED AMERSHAM PLC et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19/07/2000	06.02.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Luis Alves, D Telephone No. +49 89 2399 8695

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/04395

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-5	as originally filed

Claims, No.:

1-9	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:
   - ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2-4, 8, 9
	No:	Claims	1, 5-7
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-9
Industrial applicability (IA)	Yes:	Claims	1-9
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

Reference is made to the following document cited in the International search report:

D1: WO-A-97/37239

Section V:

1. D1 discloses the use of hyperpolarised Xe in NMR. The samples to be analysed can comprise for example proteins (see p.14, line 16 to p.15, line 4 and example 9). Thus, the subject-matter of claims 1 and 5 is not novel (Article 33(2) PCT).
D1 further discloses the use of Xe enriched at 80 % (see p.5, lines 28 to 33) and polarisation levels of 5 to 10% (see p.25, lines 18 to 19). Thus, the subject-matter of claims 6 and 7 is not novel (Article 33(2) PCT).
D1 also indicates that pressures of several atmospheres should be used (see p.25, lines 20 to 28). Thus, although the subject-matter of claim 9 is novel with respect to D1, because D1 does not disclose the specific pressure values, it does not seem to involve an inventive step (Article 33(3) PCT).
The application of the method for the analysis of the analyte in a sample, which is the object of claims 2 to 4, appears to be obvious in view of D1.
The choice of particular solvent as defined in claim 8 is neither disclosed nor suggested in any of the available documents. However, the presence of an inventive step (Article 33(3) PCT) cannot be acknowledged because the feature in claim 8 appears to be arbitrary and not to provide a solution to any technical problem.

Section VII:

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in document D1 is not mentioned in the description, nor is this document identified therein.

PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
PA9847		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/ 04395	23/12/1999	30/12/1998
Applicant		
NYCOMED AMERSHAM PLC et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 2 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. --

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/04395

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/58 C12Q1/68 G01R33/465

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C12Q G01R

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 37239 A (LAWRENCE BERKELEY NATIONAL LABORATORY) 9 October 1997 (1997-10-09) page 14, line 16 -page 15, line 4; claims 1,3,5,6,19-21; examples 2,8	1,5
A	EP 0 620 447 A (PRAXAIR TECHNOLOGY, INC.) 19 October 1994 (1994-10-19) example 1	1,5
A	WO 98 30918 A (NYCOMED IMAGING AS) 16 July 1998 (1998-07-16)	
A	WO 95 27438 A (THE RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK ET AL.) 19 October 1995 (1995-10-19)	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 April 2000	20/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Griffith, G

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/04395

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9737239 A	09-10-1997	AU 2426697 A	22-10-1997
		CA 2250401 A	09-10-1997
		EP 0890114 A	13-01-1999
		FI 982069 A	10-11-1998
		NO 984510 A	27-11-1998
EP 620447 A	19-10-1994	US 5357959 A	25-10-1994
		BR 9401499 A	13-12-1994
		CA 2121377 A	17-10-1994
		CN 1093893 A	26-10-1994
		IL 109281 A	06-12-1998
		JP 2807964 B	08-10-1998
		JP 6319721 A	22-11-1994
		KR 177532 B	01-04-1999
WO 9830918 A	16-07-1998	AU 5335298 A	03-08-1998
		EP 0951650 A	27-10-1999
WO 9527438 A	19-10-1995	US 5545396 A	13-08-1996
		AU 709515 B	02-09-1999
		AU 2278795 A	30-10-1995
		CA 2183740 A	19-10-1995
		EP 0754009 A	22-01-1997
		JP 10501708 T	17-02-1998
		US 5789921 A	04-08-1998
		US 5785953 A	28-07-1998

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WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
G01N 33/58, C12Q 1/68, G01R 33/465

A1

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(21) International Application Number: PCT/GB99/04395

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(30) Priority Data:
9828853.3 30 December 1998 (30.12.98) GB

(71) Applicant *(for all designated States except US)*: NYCOMED AMERSHAM PLC [GB/GB]; Amersham Laboratories, White Lion Road, Amersham, Bucks HP7 9LL (GB).

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: NMR SPECTROSCOPY METHOD

(57) Abstract

The invention relates to an *in vitro* method which comprises labelling a biological molecule with hyperpolarised xenon, and observing a magnetic resonance spectrum and/or image of the hyperpolarised xenon in the environment of the biological molecule. The spectrum/image provides information about the environment(s) at which atoms of xenon are bound to the biological molecule.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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NMR SPECTROSCOPY METHOD

This invention is concerned with nuclear magnetic resonance (NMR) spectroscopy and/or NMR imaging. The technique involves observing the spectrum/image of a NMR active nuclear species *in vitro* in order to obtain information about the environment in which the species is present. The spectra of NMR active nuclei vary depending on their environment, and this is reported in the literature (PNAS, 93,12932-6, 1996).

Noble gases having non-zero nuclear spin can be hyperpolarised, i.e. have their polarisation enhanced over the equilibrium polarisation, e.g. by the use of circularly polarised light. Preferred techniques for hyperpolarisation include spin exchange with an optically pumped alkali metal vapour and metastability exchange. Noble gases to which this technique can be applied include ^{3}He and ^{129}Xe. As described by M S Albert *et al* in US Patent 5,545,396, the technique can be used to prepare hyperpolarised noble gases that can be administered by inhalation for magnetic resonance imaging of the human body.

Xenon is chemically inert and has hydrophobic properties, and is capable of being weakly bound by hydrophobic regions of biological molecules (PNAS, 78, No 8, 4946-9, August 1981; Abstracts of the 11th Annual Meeting of the Society for Magnetic Resonance in Medicine (1992) page 2104). Thus it is possible to "label" biological molecules with xenon.

This invention concerns the method of labelling biological molecules with hyperpolarised ^{129}Xe. All macromolecules have a number of discrete hydrophobic and hydrophilic sites. Xenon will bind by hydrophobic interactions to hydrophobic sites with different affinity. The xenon labels the biological compound by becoming weakly bound to it, e.g. at specific hydrophobic sites on a surface of or within a cavity of a protein or other macromolecule.

The NMR sensitivity of hyperpolarised xenon is highly increased compared to non-hyperpolarised xenon. Another advantage of the present invention is the reversible and non-destructive nature of the bond between the xenon and the biological molecule. A further advantage is that the forming of the "bond" and subsequent measurement may be repeated if

needed. In addition, since xenon is a gas (condensation temperature of -106°C), it and may easily and rapidly be separated from the biological molecule if necessary. Moreover, xenon is essential chemically inert and will not adversely effect the biological molecule.

One embodiment of the invention thus provides an *in vitro* method which comprises labelling a biological molecule with hyperpolarised xenon, and observing a magnetic resonance spectrum and/or image of the hyperpolarised xenon in the environment of the biological molecule. The spectrum/image provides information about the environment(s) at which atoms of xenon are bound to the biological molecule. Any conformational change of the biological molecule resulting e.g. from the binding (or the disappearance) of a ligand (e.g. a lipid, carbohydrate, peptide, polypeptide, nucleic acid or any sort of drug) or cleavage by an enzyme, will cause an alteration in the xenon NMR spectrum. Each hydrophobic site in the biological molecule may give rise to a specific and characteristic NMR shift.

A further embodiment of the present invention is to take NMR "fingerprint(s)" of a known biological molecule. These fingerprints can subsequently be used to identify unknowns by direct comparison in a manner similar to infra-red spectroscopy.

A biological molecule as defined by the present invention is a monomeric or polymeric molecule that is present in biological systems or that is artificially introduced and is biologically active in such systems. Biological molecules include lipids, sugars and polysaccharides, nucleic acids (DNA, RNA), nucleosides, oligonucleosides, polynucleosides, nucleotides, oligonucleotides, polynucleotides, enzymes, vitamins and particularly peptides, polypeptides and proteins.

In one preferred embodiment of the invention, the labelled biological molecule is an assay reagent taking part in an assay method and wherein the assay reagent is labelled with hyperpolarised xenon. The labelling of the biological molecule with hyperpolarised xenon may be performed before, during or after performance of the assay.

An assay method according to the present invention is a test involving a reaction of one or more biological molecules. The assays include for example competition assays (e.g. receptor-

ligand antagonism, enzyme-substrate inhibitors, protein-protein interaction inhibitors), binding assays (e.g. receptor-ligand agonism, enzyme-substrate reactions, protein-protein interactions), immunoassays (e.g. for specific analytes), hybridisation assays (e.g. nuclease assays, mutation analysis, mRNA and DNA detection), test involving cells, organs and/or whole organisms. These tests may involve e.g. one or more lipids, saccharides, polynucleotides, oligonucleotides, nucleotides, peptides or proteins. Assays include binding studies performed on eukaryotic and prokaryotic microorganisms; binding studies performed on tissue *in vitro*; and binding studies in which an assay reagent is administered *in vivo* and an excretion product (e.g. urine, faeces, or breath) analysed by NMR *in vitro*.

By observing a change with time using NMR, the progress of a reaction can be followed during the course of an assay. Assays performed *in vitro* may conveniently be in multiwell plates, with either an assay reagent in the wells of the plate being labelled with hyperpolarised xenon, or a reagent being so labelled in bulk prior to being dispensed into individual wells of the plate.

Generally the biological molecule is present in a liquid medium into which the xenon is introduced as a gas. This may be achieved e.g. by bubbling it through the fluid or by contact with the biological molecule as a solid. Alternatively the xenon is introduced as a solution in a solvent, which is compatible with the biological molecule (e.g. in a lipophilic solvent such as a lipid or a fluorocarbon solvent).

The liquid medium used according to one embodiment of the present invention may be deuterated water, deuterated buffers or solvents, e.g. lipophilic solvents which may contain lipid bicelles, lipid vesicles, liposomes, cryptophanes and/or cyclodextrins.

^{129}Xe has a natural abundance of 26.4%. The xenon used for this invention may be either the naturally occurring material or one artificially enriched in ^{129}Xe. A preferred degree of enrichment ^{129}Xe is 40 % or more. A more preferred degree is 50 % or more and an even more preferred degree is 75 % or more. A particularly preferred degree of enrichment is 90 % or more. Bulk supplies of xenon enriched in ^{129}Xe and hyperpolarised to a high degree are now available commercially and have a half life long enough to permit transport over substantial

distances. While the half life of hyperpolarised ^{129}Xe in the biological environments contemplated in this invention will be lower, it is expected to be amply sufficient to permit the desired spectra to be obtained. A preferred degree of hyperpolarisation is 8 % or more. A more preferred hyperpolarisation degree is 20 % or more and an even more preferred degree is 30 % or more. Ideally, the degree will approach 100 %.

In one embodiment of the invention, the temperature at the time xenon is added is above the temperature at which the biological molecule is frozen, but below the temperature at which the biological molecule may be denatured. Alternatively, xenon may be added to the frozen biological molecule, followed by thawing. However, the right temperature to achieve the optimal function of the biological molecule should also be considered.

In one embodiment of the invention, the solution is kept as low as possible in order to slow down the exchange between the bound xenon and free xenon, without broadening the NMR signals too much.

In a further embodiment of the invention, the solution is made viscous due to the use of one viscous solvent or the use of a suitable combination of solvents. The viscosity of the solvent is preferably within the range of 500 mPs to 5000 mPs, more preferably within the range of 700 mPs to 1500 mPs.

In one embodiment of the invention, the pressure of xenon is as high as possible, preferably higher than 5 $x10^5$ N/m^2 (5 bar), more preferably higher than 5 x 10^6 N/m^2 (50 bar), even more preferably higher than 1 $x10^7$ N/m^2 (100 bar) and particularly higher than $2x10^7$ N/m^2 (200 bar). However, the pressure must never be so high that the biological molecule will be adversely effected.

The invention is illustrated with reference to the following non-limiting Example:

Hyperpolarised ^{129}Xe is generated by optical pumping as described by B.Driehuys et al., Appl.Phys.Lett. 69 (12), 1996. The Isotopic composition of the gas is 80% ^{129}Xe and 0.25% ^{131}Xe (the rest non-magnetic isotopes of Xe). The degree of polarisation is estimated to be 10%.

Lyzozyme (28 mg) is dissolved in a mixture of D_2O and methanol-d_4 (40:60) (3 ml) in a heavy-walled 10 mm NMR-tube. This mixture is subjected to four freeze-pump-thaw cycles of degassing. The tube is then connected to the outlet of the polariser and frozen in liquid nitrogen. The hyperpolarized gas is generated and collected on a cold finger at liquid nitrogen temperature in a holding field of 200 mT over a period of 15 minutes which is estimated to give a volume of 50 ml of Xe at NTP. A narrow Dewar vessel with liquid nitrogen is placed in a magnet with a field strength of 0.3 T. The collected xenon is thawed and then refrozen in the NMR-tube in the 0.3 T magnet. The sample tube is flame-sealed and the frozen sample is moved to the fringe field of the magnet of an NMR-spectrometer. The NMR-spectrometer sample space is kept at a temperature of 293 K. The sample is removed from the transport magnet and thawed by heating with the hand (protected from the cold) while standing as close to the NMR-magnet as possible. When the sample starts to thaw it is shaken vigorously and inserted into the spectrometer. A ^{129}Xe spectrum is recorded and apart from the large peak due to the bulk xenon, a small peak, with a line width of 160 Hz, due to bound xenon can be observed at -158 ppm relative to bulk xenon.

CLAIMS

1. An *in vitro* method which comprises labelling a biological molecule with hyperpolarised ^{129}Xe, and observing a magnetic resonance (NMR) spectrum and/or NMR image of the hyperpolarised ^{129}Xe in the environment of the biological molecule.

2. The method of claim 1 wherein the biological molecule is an assay reagent taking part in an assay method.

3. The method of claim 2 wherein the assay is a competition assay or an immunoassay.

4. The method of claim 2 wherein the assay is a hybridisation assay or a binding assay.

5. The method of any of claims 1 to 4 wherein the biological molecule is a peptide or a protein.

6. The method of any of claims 1 to 5 wherein the hyperpolarised ^{129}Xe is enriched at a level of 40 % or more.

7. The method of any of claims 1 to 6 wherein the degree of hyperpolarisation is 8 % or more.

8. The method of any of claims 1 to 7 which is performed in a solution wherein the solvent has a viscosity in the range of 700 to 1500 mPs.

9. The method of any of claims 1 to 8 wherein the pressure of the xenon gas is at least 5 bar.

INTERNATIONAL SEARCH REPORT

Inte ..ional Application No
PCT/GB 99/04395

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/58 C12Q1/68 G01R33/465

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C12Q G01R

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 37239 A (LAWRENCE BERKELEY NATIONAL LABORATORY) 9 October 1997 (1997-10-09) page 14, line 16 -page 15, line 4; claims 1,3,5,6,19-21; examples 2,8	1,5
A	EP 0 620 447 A (PRAXAIR TECHNOLOGY, INC.) 19 October 1994 (1994-10-19) example 1	1,5
A	WO 98 30918 A (NYCOMED IMAGING AS) 16 July 1998 (1998-07-16)	
A	WO 95 27438 A (THE RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK ET AL.) 19 October 1995 (1995-10-19)	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 April 2000	20/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Griffith, G

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ..ional Application No
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